Published in final edited form as:

Behav Brain Res. 2018 January 30; 337: 246–251. doi:10.1016/j.bbr.2017.09.014.

ACID-SENSING ION CHANNEL 1 CONTRIBUTES TO NORMAL OLFACTORY FUNCTION

Kiara T. Vann¹ and Zhi-Gang Xiong*

¹Department of Neurobiology, Morehouse School of Medicine, Atlanta, Georgia

Abstract

Acid-sensing ion channels (ASICs) are cation channels activated by protons. ASIC1a, a primary ASIC subunit in the brain, was recently characterized in the olfactory bulb. The present study tested the hypothesis that ASIC1a is essential for normal olfactory function. Olfactory behavior of wild-type (WT) and ASIC1^{-/-} mice was evaluated by using three standard olfactory tests: (1) the buried food test, (2) the olfactory habituation test, and (3) the olfactory preference test. In buried food test, ASIC1^{-/-} mice had significantly longer latency to uncover buried food than WT mice. In olfactory habituation test, ASIC1^{-/-} mice had increased sniffing time with acidic odorants. In olfactory preference test, ASIC1^{-/-} mice did not exhibit normal avoidance behavior for 2, 5-dihydro-2, 4, 5-trimethylthiazoline (TMT). Consistent with ASIC1 knockout, ASIC1 inhibition by nasal administration of PcTX1 increased the latency for WT mice to uncover the buried food. Together, these findings suggest a key role for ASIC1a in normal olfactory function.

Graphical abstract

Keywords

ASICs; Olfactory Behavior; Buried Food Test; Olfaction

*Corresponding Author. Mailing address: 720 Westview Drive S.W., MRC215, Atlanta, GA 30310. Phone: (404) 752-8683. Fax: (404) 752-1041. zxiong@msm.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Acid-Sensing Ion Channels (ASICs) are members of the Degenerin/Epithelial Na⁺ channel (DEG/ENaC) superfamily [1–5]. Reductions in extracellular pH activate these channels in neurons of the central nervous systems (CNS) and peripheral sensory systems. ASICs respond to extracellular pH drop (~7.0 or lower) by conducting transient inward currents, which causes membrane depolarization and neuronal excitation. Currently, there are six known isoforms: ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3 and ASIC4 [6–8].

ASIC1a, ASIC2a and ASIC2b are predominant ASIC subunits located in the majority of CNS neurons [8–10]. ASIC1a subunits are abundant in the cerebral cortex, hippocampus, and olfactory bulb [2,11,12]. Activation of ASIC1a has been demonstrated to play an important role in learning/memory and fear conditioning [11,12]. Other potential functions have yet to be determined. The olfactory bulb is responsible for sending olfactory information to the amygdala, orbitofrontal cortex and the hippocampus for further processing [13]. The electrophysiological and pharmacological properties of ASICs in the mitral/tufted cells of the olfactory bulb have been well characterized in our recent study [14]. However, the involvement of ASICs in olfactory behavior remains unknown. In the present study, we evaluated the performance of *ASIC1^{-/-}* and wild-type (WT) mice in commonly used behavioral paradigms that assess the function of olfactory system. Our data strongly suggest that ASIC1a plays an important role in normal olfactory function.

2. Materials and Methods

2.1 Animals

C57BL/6 mice were purchased from Charles River Laboratory (Wilmington, MA) and *ASIC1^{-/-}* mice with a generic C57BL/6 background were generated in house [11,15]. In general, male mice at least eight weeks of age were used. Mice were housed with a 12-h light-dark cycle and *ad libitum* access to food and water except for fasting period during buried food-tests. All experimental procedures were approved by Morehouse School of Medicine Institutional Animal Care and Use Committee. All animals were treated humanely in accordance with the *Guide for the Care and Use of Laboratory Animals*.

2.2 Chemicals/Compounds

Zinc gluconate was purchased from Fisher Scientific (Waltham, MA) and dissolved in deionized water with a final concentration of 33 mM, as described in previous studies [16]. PcTX1 was manufactured by Peptide International (Louisville, KY) and diluted to a final concentration of 1 μ M in PBS. 2, 5-dihydro-2, 4, 5-trimethylthiazoline (TMT) was purchased from Sigma-Aldrich (St. Louis, MO) and diluted in deionized water to a final concentration of 8%, the concentration commonly used in the literature [17]. Peanut butter extract was purchased from J.R. Watkins (Winona, MN) and diluted to a final concentration of 8%.

2.3 Behavioral Testing

2.3.1 Buried food Test—*ASIC1*^{-/-} and WT mice, at least eight weeks of age, were used for the buried food test (BFT) to detect olfactory impairments [18,19]. Two days before the test, ~1.5 g of Elfin Crackers (Kellogg's, Battle Creek, NJ) was placed in the cage to determine if the food was palatable to the mice. All mice underwent an overnight fasting before testing. The location of the food was randomly placed daily. The latency to locate food was defined as the time between when the mouse was placed in the cage and when the mouse uncovered the food and grasped it in its forepaws and/or teeth. Animals were allowed to consume the food they uncovered and were returned to their home cage after testing. Mice that failed to uncover food within 10 minutes were scored with a latency of 600 seconds. The bedding in the testing cage was changed between trials and animals. The surface pellet test was identical to the buried food test except that the food was visible.

2.3.2 Olfactory Habituation/Dishabituation Test—*ASIC1*^{-/-} and WT mice, at least eight weeks of age, were used to detect odor discrimination. Non-social odors (i.e. distilled water, pure orange extract and acetic acid (J.R. Watkins, Winona, MN) were used for the present studies. Odorants were diluted to final concentration of 1:1000 in distilled water [20–23]. Mice were allowed a 30 minute acclimation period before testing. The test was performed by inserting the cotton tipped applicator into the cage lid, and the cumulative time that mice spent sniffing the tip was recorded during the 2 minute period. Sniffing was timed when the mouse orients towards the cotton-tipped applicator with its nose approximately 2 mm to the tip. Once a trial was completed, the cage lid was removed, and the applicator was changed to start the next trial.

2.3.3 Olfactory Preference Test—*ASIC1*^{-/-} and WT mice, at least eight weeks of age, were used to determine which odorants are perceived as an attractant or repellant [23–25]. Mice were allowed a 10 minute acclimation period before testing. 50 µl of an attractant odor (peanut butter extract) and 50 µl of the neutral scent (tap water) was pipetted onto the bedding next to a wall away from the mouse. The exploratory behavior was recorded within a 2 minute time frame on a video camera. The avoidance test was performed in the same manner by applying 50 µl of the repellant odor (TMT) and 50 µl of water.

2.4 Zinc Gluconate or PcTX1 Nasal Irrigation

Zinc gluconate treatment began an hour after baseline behavioral analysis using the BFT. Mice were held with proper restraint [16,26] and 50 µl of 33 mM zinc gluconate solution was administered approximately 2 mm into the mouse's left nostril under light isoflurane anesthesia. About 15 minutes later, the same solution was administered into the right nostril. Experimentation began 1 hour after treatment. Control mice were nasally irrigated with PBS.

2.5 ASIC inhibition

PcTX1 treatment began an hour after baseline behavioral analysis using the BFT. Mice were held with proper restraint and received intranasal irrigation of ASIC1 inhibitor, PcTX1 (50 µl, 1 µM) or PBS. Experimentation began 1 hour after treatment.

2.6 Data Analysis

Data are expressed as the mean $\pm$ SEM. ANOVA followed by a Newman-Keuls post-hoc test was used for statistical analysis. All statistical analysis was performed using Sigma Plot 12.0 and $p < 0.05$ is considered as statistically significant.

3. Results

3.1 Buried food test

In the present study, we first explored whether WT and *ASIC1*^{-/-} mice differ in their capability to locate food. The BFT is a commonly used and reliable procedure that relies on the innate ability of the mouse to use olfactory cues for foraging [18,19]. The main parameter is the latency to uncover palatable food, i.e. cookie, hidden beneath the bedding, within a given timeframe. We first tested the palatability of food, by leaving the cookie with mice overnight (see Materials and Methods section) and observed no difference between WT and *ASIC1*^{-/-} mice, i.e., both groups consumed the whole cookie.

Both *ASIC1*^{-/-} and WT mice found the buried food in less than 100 s over the trials, however *ASIC1*^{-/-} mice were slower at finding the buried food compared to WT mice. The latency for trial 1 was significantly longer for *ASIC1*^{-/-} mice as compared to WT mice (68 ± 12.3 s for *ASIC1*^{-/-} vs. 33.2 ± 6.8 s for WT mice, $p < 0.05$, $n = 8-9$, Figure 1A). Within genotypes, *ASIC1*^{-/-} mice showed some improvement in their ability to find the food throughout 3 trials while WT mice, on the other hand, had similar latency throughout 3 trials. These findings suggest that *ASIC1*^{-/-} mice have some deficit in uncovering buried food. Pooling the data of 3 trials together, we observed a two-fold increase in the latency of *ASIC1*^{-/-} mice to find the buried food (55.1 ± 5.7 s vs 32.8 ± 3.5 s; $p < 0.01$ (Figure 1B). Additionally, we conducted a surface pellet test. Surprisingly, we observed a significant two-fold increase in the latency to find visible food (17.7 ± 2.9 s vs 8.8 ± 2.2 s; $p < 0.05$) in *ASIC1*^{-/-} mice compared to WT mice. This finding also suggests that *ASIC1*^{-/-} mice may have difficulties in perceiving emitted odors.

3.2 Olfactory habituation/dishabituation test

To further assess the deficit in olfactory function of *ASIC1*^{-/-} mice, we used the habituation/dishabituation test, which relies on animal's ability to explore novel odors and to distinguish between different odors. Both genotypes were able to habituate to different odors after repeated exposures (Figure 2A–C). However, compared to WT mice, *ASIC1*^{-/-} mice showed a significant increase in their investigation time to orange extract (8.5 ± 1.7 s vs 3.4 ± 1.1 s; $p < 0.01$) and acetic acid (2.9 ± 1.4 s vs 0.68 ± 0.5 s; $p < 0.01$), respectively (Figure 2B–C). These findings further suggest that *ASIC1*^{-/-} mice have impairments in their odor sensitivity.

3.3 Olfactory preference test

We next examined whether *ASIC1*^{-/-} mice have impairments in their ability to perceive odors as pleasant or aversive. We used the olfactory preference test, which measures whether animals spend significantly longer time investigating an odorant versus water (attraction) or significantly less time versus water (avoidance). In this study, mice were tested for their

investigative behavior for peanut butter or 2, 5-dihydro- 2,4,5-trimethylthiazoline (TMT). TMT is a synthetic compound derived from fox feces that induces innate fear and stress responses among rodents [27,28]. Both genotypes showed attractive behavior for peanut butter and no significant differences in investigation time were observed (1.6–2s, Figure 3A). In the innate olfactory avoidance test (Figure 3B), however, *ASIC1*^{-/-} mice had ~ 4-fold increase in their investigation time with TMT compared to WT mice (0.55 ± 0.2 vs. 0.14 ± 0.1 s; $p < 0.05$).

3.4 Effect of nasal irrigation of PcTX1 on olfactory function

To further determine the role of ASIC1 in olfactory function, we studied the effect of nasal administration of PcTX1, an inhibitor of ASIC1a channels [27–29], on olfactory function using the buried food test.

As a positive control, we first tested the effect of nasal administration of zinc gluconate, an agent known to inhibit olfactory function or cause anosmia (loss of smell) [16,30]. 1 hr after nasal administration, zinc gluconate-treated mice displayed a dramatic increase in the latency for uncovering the buried food (Figure 4). A significant difference was observed between experimental group (417.1 ± 110.1 s) vs baseline performance (59.3 ± 13.6 s; $p < 0.05$). 1h after nasal administration, PcTX1-treated mice also displayed an increase in the latency for uncovering the buried food (Figure 5). The mean latency for experimental group (145.3 ± 21.5 s) was significant higher than baseline performance (83.3 ± 8.7 s; $p < 0.05$).

4. Discussion and conclusion

In the present study, we examined whether ASIC1a plays a role in normal olfactory behavior/function. We first used *ASIC1*^{-/-} mice to evaluate the function of ASIC1a in the olfactory system by performing different olfactory behavioral tests that included buried food, olfactory habituation/dishabituation and olfactory preference. The buried food test examines the mouse's ability to identify and locate palatable food using olfactory cues, whereas, in the habituation/dishabituation test, mice were exposed to different non-social odors to evaluate their ability to detect and differentiate odors. The olfactory preference test examines the ability of mice to recognize attractive or aversive odors. Lastly, zinc gluconate and PcTX1 were applied through intranasal administration to evaluate their effects on the outcome of BFT. A key finding of this study is that *ASIC1*^{-/-} mice performed poorly in all olfactory paradigms examined compared to WT mice.

Mice can be evaluated for different aspects of olfactory functions including odor discrimination, odor threshold, and odor identification [31]. Unlike humans, rodents have two distinct olfactory pathways: the main olfactory system and the accessory olfactory system. Both are important in the detection of odors, having completely different but overlapping roles. The main olfactory system is vital in the detection of odors in the environment and the accessory olfactory system plays a key role in pheromone recognition [32]. Odor detection is highly associated with olfactory threshold. Hummel et al found that odor threshold declines drastically with age compared to other odor modalities [33]. In our study, we first used a simple and common method to test olfactory detection, the buried food test. The finding that *ASIC1*^{-/-} mice took a significant longer time in uncovering the buried

food provided the initial evidence that ASIC1 plays a role in olfactory detection. We then used olfactory habituation/dishabituation test to further explore the difference between WT and ASIC1^{-/-} mice. The deficits in habituation/dishabituation test were clearly seen in ASIC1^{-/-} mice as indicated by an increase in investigation time compared to WT mice. Since a single sniff of an odorant is sufficient to cause a complex odor detection and discrimination, it has been suggested that a prolonged sniffing is a sign of deficits in odor discrimination and/or detection [34].

Rodents display innate avoidance behavior towards predators' odors [35–38] and they also avoid spoiled food smells [39], such as aliphatic acids [36,38], aliphatic aldehydes [40] and alkyl amines [41]. Conversely, rodents show attractive behaviors toward food smells and conspecific odors [42]. ASIC1^{-/-} mice did not find aliphatic acids, specifically acetic acid and pure orange extract to be offensive smells. Based on this, we used olfactory preference test to provide further assessment of mouse's olfactory functions, in addition to odor detection and discrimination tests. We found that ASIC1^{-/-} mice failed to exhibit normal avoidance behavior for TMT, as evidenced by a significant longer investigation time compared to WT mice. These findings further support the notion that ASIC1^{-/-} mice have some form of olfactory impairments.

Intranasal application of zinc sulfate is a widely accepted method to produce anosmia in olfactory studies. While it most likely affects odor transduction, the mechanism has not been fully elucidated. Some studies have suggested zinc effect on olfactory epithelium and that high concentrations (10–30 μ m) can be neurotoxic [43–47]. Other studies have shown that zinc gluconate is effective in causing anosmia without the detrimental effects of zinc sulfate [16,30,48]. Interestingly, zinc has been shown to inhibit the functions of ASIC1a containing channels, which action might have contributed to its inhibition of the olfactory function [49]. Nevertheless, in our study, mice that received intranasal applications of zinc gluconate showed a dramatic increase in the latency of uncovering the buried food.

Consistent with ASIC1 knockout, intranasal administration of ASIC1 inhibitor PcTX1 to wild-type mice resulted in olfactory deficits: PcTX1-treated animals had a longer latency in uncovering the buried food compared with animals treated with PBS. The present study mainly focuses on the involvement of ASIC1a in olfactory function. Future studies may determine how irrigation of broad ASIC inhibitors such as amiloride or diminazene [50] affects the olfactory function.

The mechanism whereby ASIC1a deficiency leads to olfactory impairments in mice is unknown. Waldmann et al. have demonstrated exceptionally high levels of ASIC1a mRNA in the olfactory bulb [2]. Immunolabeling studies have shown particularly prominent ASIC1a expression in the olfactory tubercle, glomerular layer and within the glomeruli [12]. Our previous studies revealed the presence of ASIC1a, ASIC2a and ASIC3 in the mitral/ tufted cells of the olfactory bulb and characterized the electrophysiological and pharmacological properties of these channels [14]. In addition, ASIC1a is also expressed in brain regions such as hippocampus, amygdala and piriform cortex that are important for higher level odor processing [11,12,51]. These regions are important for odor identification, odor memory and odor-induced behavior [52,53]. Additional studies need to be performed to

examine ASIC1a expression in cortical areas that process olfactory information such as the orbital frontal cortex, anterior olfactory nucleus, and the entorhinal cortex [54].

How ASIC1a contributes to olfaction requires further investigation. It is not clear whether ASIC1a functions through the canonical adenylyl cyclase 3 mediated-cAMP olfactory signaling pathway or via a direct membrane depolarization of the olfactory sensory neurons. Nevertheless, the present study disclosed an important function of ASIC1a in normal olfactory function. The underlying mechanism has yet to be determined.

Acknowledgments

The authors want to thank Drs. Todd White and Zaven O'Bryant for proofreading the manuscript. This work was supported by the National Institutes of Health (R01NS066027, S21MD000101 and U54NS08932) to Zhi-Gang Xiong and Research Initiative for Scientific Enhancement Program (RISE 5R25 GM058268) to Kiara T. Vann. The funding sources had no input in the design of the study and collection, analysis, and interpretation of data and writing the manuscript.

Abbreviations

WT	wild-type
TMT	2,5-dihydro-2,4,5-trimethylthiazoline
ASICs	Acid-Sensing Ion Channels
DEG/ENaC	Degenerin/Epithelial Na ⁺ channel
CNS	central nervous system
BFT	buried food test

References

1. Bassilana F, Champigny G, Waldmann R, de Weille JR, Heurteaux C, Lazdunski M. The Acid-sensitive Ionic Channel Subunit ASIC and the Mammalian Degenerin MDEG Form a Heteromultimeric H⁺-gated Na⁺ Channel with Novel Properties. *J Biol Chem.* 1997; 272:28819–28822. DOI: 10.1074/jbc.272.46.28819 [PubMed: 9360943]
2. Waldmann R, Champigny G, Bassilana F, Heurteaux C, Lazdunski M. A proton-gated cation channel involved in acid-sensing. *Nature.* 1997; 386:173–177. DOI: 10.1038/386173a0 [PubMed: 9062189]
3. Garcia-Anoveros J, Derfler B, Neville-Golden J, Hyman BT, Corey DP. BNaC1 and BNaC2 constitute a new family of human neuronal sodium channels related to degenerins and epithelial sodium channels. *Proc Natl Acad Sci.* 1997; 94:doi: 10.1073/pnas.94.4.1459
4. Krishtal O. The ASICs: Signaling molecules? Modulators? *Trends Neurosci.* 2003; 26:477–483. DOI: 10.1016/S0166-2236(03)00210-8 [PubMed: 12948658]
5. O'Bryant Z, Vann KT, Xiong Z-G. Translational strategies for neuroprotection in ischemic stroke - focusing on Acid Sensing Ion Channel 1a. *Transl Stroke Res.* 2014; 5:59–68. DOI: 10.1007/s12975-013-0319-5 [PubMed: 24390970]
6. Akopian AN, Chen CC, Ding Y, Cesare P, Wood JN. A new member of the acid- sensing ion channel family. *NeuroReport.* 2000; 11 http://journals.lww.com/neuroreport/Fulltext/2000/07140/A_new_member_of_the_acid_sensing_ion_channel.31.aspx.
7. Gründer S, Chen X. Structure, function, and pharmacology of acid-sensing ion channels (ASICs): focus on ASIC1a. *Int J Physiol Pathophysiol Pharmacol.* 2010; 2:73–94. [PubMed: 21383888]

8. Sherwood TW, Frey EN, Askwith CC. Structure and activity of the acid-sensing ion channels. *Am J Physiol - Cell Physiol.* 2012; 303:C699–C710. DOI: 10.1152/ajpcell.00188.2012 [PubMed: 22843794]
9. de la Rosa DA, Krueger SR, Kolar A, Shao D, Fitzsimonds RM, Canessa CM. Distribution, subcellular localization and ontogeny of ASIC1 in the mammalian central nervous system. *J Physiol.* 2003; 546:77–87. DOI: 10.1113/jphysiol.2002.030692 [PubMed: 12509480]
10. Waldmann R, Bassilana F, de Weille J, Champigny G, Heurteaux C, Lazdunski M. Molecular Cloning of a Non-inactivating Proton-gated Na⁺ Channel Specific for Sensory Neurons. *J Biol Chem.* 1997; 272:20975–20978. DOI: 10.1074/jbc.272.34.20975 [PubMed: 9261094]
11. Wemmie JA, Chen J, Askwith CC, Hruska-Hageman AM, Price MP, Nolan BC, Yoder PG, Lamani E, Hoshi T, Freeman JH Jr, Welsh MJ. The Acid-Activated Ion Channel ASIC Contributes to Synaptic Plasticity, Learning, and Memory. *Neuron.* 2002; 34:463–477. DOI: 10.1016/S0896-6273(02)00661-X [PubMed: 11988176]
12. Wemmie JA, Askwith CC, Lamani E, Cassell MD, Freeman JH, Welsh MJ. Acid-Sensing Ion Channel 1 Is Localized in Brain Regions with High Synaptic Density and Contributes to Fear Conditioning. *J Neurosci.* 2003; 23:5496. [PubMed: 12843249]
13. Royet JP, Plailly J. Lateralization of Olfactory Processes. *Chem Senses.* 2004; 29:731–745. DOI: 10.1093/chemse/bjh067 [PubMed: 15466819]
14. Li MH, Liu SQ, Inoue K, Lan J, Simon RP, Xiong ZG. Acid-sensing ion channels in mouse olfactory bulb M/T neurons. *J Gen Physiol.* 2014; 143:719–731. DOI: 10.1085/jgp.201310990 [PubMed: 24821964]
15. Price MP, Snyder PM, Welsh MJ. Cloning and expression of a novel human brain Na-channel. *J Biol Chem.* 1996; 271:7879–7882. DOI: 10.1074/jbc.271.14.7879 [PubMed: 8626462]
16. Duncan-Lewis CA, Lukman RL, Banks RK. Effects of Zinc Gluconate and 2 Other Divalent Cationic Compounds on Olfactory Function in Mice. *Comp. Med.* 61. 2011:361–365.
17. Endres T, Apfelbach R, Fendt M. Behavioral Changes Induced in Rats by Exposure to Trimethylthiazoline, a Component of Fox Odor. *Behav Neurosci.* 2005; 119:1004–1010. DOI: 10.1037/0735-7044.119.4.1004 [PubMed: 16187828]
18. Yang M, Crawley JN. Simple Behavioral Assessment of Mouse Olfaction. *Curr Protoc Neurosci.* 2009; Editor Board Jacqueline N Crawley AI. CHAPTER. Unit-8.24. doi: 10.1002/0471142301.ns0824s48
19. Crawley, JN. Behavioral Phenotyping of Transgenic and Knockout Mice. Hoboken, New Jersey: John Wiley and Sons; 2007. What's Wrong with my Mouse?; p. 42-60.n.d.
20. Nathan BP, Yost J, Litherland MT, Struble RG, Switzer PV. Olfactory function in apoE knockout mice. *Behav Brain Res.* 2004; 150:1–7. [PubMed: 15033273]
21. Chaudhury D, Manella L, Arellanos A, Escanilla O, Cleland TA, Linster C. Olfactory bulb habituation to odor stimuli. *Behav Neurosci.* 2010; 124:490. [PubMed: 20695648]
22. Slotnick B, Schellinck H. Methods in olfactory research with rodents. *Front Methods Chemosenses* CRC Press N Y. 2002:21–61.
23. Zou J, Wang W, Pan YW, Lu S, Xia Z. Methods to measure olfactory behavior in mice. *Curr Protoc Toxicol.* 2015; :11.18.1–11.18.21. Editor. Board Mahin Maines Ed.–Chief AI. 63. DOI: 10.1002/0471140856.tx1118s63
24. Kobayakawa K, Kobayakawa R, Matsumoto H, Oka Y, Imai T, Ikawa M, Okabe M, Ikeda T, Itohara S, Kikusui T, Mori K, Sakano H. Innate versus learned odour processing in the mouse olfactory bulb. *Nature.* 2007; 450:503–508. DOI: 10.1038/nature06281 [PubMed: 17989651]
25. Witt RM, Galligan MM, Despinoy JR, Segal R. Olfactory Behavioral Testing in the Adult Mouse. *J Vis Exp JoVE.* 2009; :949.doi: 10.3791/949 [PubMed: 19229182]
26. Machholz E, Mulder G, Ruiz C, Corning BF, Pritchett-Corning KR. Manual Restraint and Common Compound Administration Routes in Mice and Rats. 2012; :e2771.doi: 10.3791/2771
27. Escoubas P, De Weille JR, Lecoq A, Diochot S, Waldmann R, Champigny G, Moinier D, Ménez A, Lazdunski M. Isolation of a Tarantula Toxin Specific for a Class of Proton-gated Na⁺ Channels. *J Biol Chem.* 2000; 275:25116–25121. DOI: 10.1074/jbc.M003643200 [PubMed: 10829030]

28. Chen X, Kalbacher H, Gründer S. The Tarantula Toxin Psalmotoxin 1 Inhibits Acid-sensing Ion Channel (ASIC) 1a by Increasing Its Apparent H(+)Affinity. *J Gen Physiol.* 2005; 126:71–79. DOI: 10.1085/jgp.200509303 [PubMed: 15955877]
29. Chen X, Kalbacher H, Gründer S. Interaction of Acid-sensing Ion Channel (ASIC) 1 with the Tarantula Toxin Psalmotoxin 1 is State Dependent. *J Gen Physiol.* 2006; 127:267–276. DOI: 10.1085/jgp.200509409 [PubMed: 16505147]
30. Slotnick B, Sanguino A, Husband S, Marquino G, Silberberg A. Olfaction and olfactory epithelium in mice treated with zinc gluconate. *The Laryngoscope.* 2007; 117:743–749. [PubMed: 17415147]
31. Eibenstein A, Fioretti AB, Lena C, Rosati N, Amabile G, Fusetti M. Modern psychophysical tests to assess olfactory function. *Neurol Sci.* 2005; 26:147–155. DOI: 10.1007/s10072-005-0452-3 [PubMed: 16086127]
32. Restrepo D, Arellano J, Oliva AM, Schaefer ML, Lin W. Emerging views on the distinct but related roles of the main and accessory olfactory systems in responsiveness to chemosensory signals in mice. *Olfaction Sex Behav.* 2004; 46:247–256. DOI: 10.1016/j.yhbeh.2004.02.009
33. Hummel T, Rissom K, Reden J, Hähner A, Weidenbecher M, Hüttenbrink KB. Effects of olfactory training in patients with olfactory loss. *The Laryngoscope.* 2009; 119:496–499. DOI: 10.1002/lary.20101 [PubMed: 19235739]
34. Kepecs A, Uchida N, Mainen ZF. The Sniff as a Unit of Olfactory Processing. *Chem Senses.* 2006; 31:167–179. [PubMed: 16339265]
35. Vernet-Maury E, Polak E, Demael A. Structure-activity relationship of stress-inducing odorants in the rat. *J Chem Ecol.* 1984; 10:1007–1018. [PubMed: 24318845]
36. Hebb AL, Zacharko RM, Dominguez H, Trudel F, Laforest S, Drolet G. Odor-Induced Variation in Anxiety-Like Behavior in Mice is Associated with Discrete and Differential Effects on Mesocorticolimbic Cholecystokinin mRNA Expression. *Neuropsychopharmacology.* 2002; 27:744–755. [PubMed: 12431849]
37. Takahashi LK. Olfactory systems and neural circuits that modulate predator odor fear. *Olfactory Mem Netw Emot Learn Soc Behav.* 2015
38. Hebb AL. Brief exposure to predator odor and resultant anxiety enhances mesocorticolimbic activity and enkephalin expression in CD-1 mice. *Eur J Neurosci.* 2004; 20:2415–2429. DOI: 10.1111/j.1460-9568.2004.03704.x [PubMed: 15525282]
39. Li Q, Liberles SD. Aversion and attraction through olfaction. *Curr Biol CB.* 2015; 25:R120–R129. DOI: 10.1016/j.cub.2014.11.044 [PubMed: 25649823]
40. Wood RW, Coleman JB. Behavioral evaluation of the irritant properties of formaldehyde. *Toxicol Appl Pharmacol.* 1995; 130:67–72. DOI: 10.1006/taap.1995.1009 [PubMed: 7839371]
41. Dielenberg RA, McGregor IS. Defensive behavior in rats towards predatory odors: a review. *Neurosci Biobehav Rev.* 2001; 25:597–609. [PubMed: 11801285]
42. Nyby J, Kay E, Bean NJ, Dahinden Z, Kerchner M. Male mouse attraction to airborne urinary odors of conspecific and to food odors; effects of food deprivation. *J Comp Psychol.* 1985; 99:479–490. DOI: 10.1037/0735-7036.99.4.479
43. Alberts JR, Galef BG Jr. Acute anosmia in the rat: A behavioral test of a peripherally-induced olfactory deficit. *Physiol Behav.* 1971; 6:619–621. DOI: 10.1016/0031-9384(71)90218-6 [PubMed: 5149455]
44. McBride K, Slotnick B, Margolis FL. Does Intranasal Application of Zinc Sulfate Produce Anosmia in the Mouse? An Olfactometric and Anatomical Study. *Chem Senses.* 2003; 28:659–670. DOI: 10.1093/chemse/bjg053 [PubMed: 14627534]
45. Trombley PQ, Horning MS, Blakemore ML. Interactions between carnosine and zinc and copper: implications for neuromodulation and neuroprotection. *Biochem Mosc.* 2000; 65:807–16.
46. Trombley PQ, Shepherd GM. Differential modulation by zinc and copper of amino acid receptors from rat olfactory bulb neurons. *J Neurophysiol.* 1996; 76:2536. [PubMed: 8899625]
47. Horning MS, Blakemore LJ, Trombley PQ. Endogenous mechanisms of neuroprotection: role of zinc, copper, and carnosine. *Brain Res.* 2000; 852:56–61. DOI: 10.1016/S0006-8993(99)02215-5 [PubMed: 10661495]
48. Jafek BW, Linschoten MRI, Murrow BW. Anosmia after Intranasal Zinc Gluconate Use. *Am J Rhinol.* 2004

49. Chu XP, Wemmie JA, Wang WZ, Zhu XM, Saugstad JA, Price MP, Simon RP, Xiong ZG. Subunit-Dependent High-Affinity Zinc Inhibition of Acid-Sensing Ion Channels. *J Neurosci*. 2004; 24:8678.doi: 10.1523/JNEUROSCI.2844-04.2004 [PubMed: 15470133]
50. Chen X, Qiu L, Li M, Dürrnagel S, Orser BA, Xiong ZG, MacDonald JF. Diarylamidines: High potency inhibitors of acid-sensing ion channels. *Neuropharmacology*. 2010; 58doi: 10.1016/j.neuropharm.2010.01.011
51. Wu PY, Huang YY, Chen CC, Hsu TT, Lin YC, Weng JY, Chien TC, Cheng IH, Lien CC. Acid-Sensing Ion Channel-1a Is Not Required for Normal Hippocampal LTP and Spatial Memory. *J Neurosci*. 2013; 33:1828.doi: 10.1523/JNEUROSCI.4132-12.2013 [PubMed: 23365222]
52. Nordin S, Murphy C. Impaired sensory and cognitive olfactory function in questionable Alzheimer's disease. *Neuropsychology*. 1996; 10:113–119. DOI: 10.1037/0894-4105.10.1.113
53. EICHENBAUM H, MORTON TH, POTTER H, CORKIN S. SELECTIVE OLFACTORY DEFICITS IN CASE H.M. *Brain*. 1983; 106:459.doi: 10.1093/brain/106.2.459 [PubMed: 6850278]
54. Wilson RS, Schneider JA, Arnold SE, Tang Y, Boyle PA, Bennett DA. Olfactory identification and incidence of mild cognitive impairment in older age. *Arch Gen Psychiatry*. 2007; 64:802–808. DOI: 10.1001/archpsyc.64.7.802 [PubMed: 17606814]

HIGHLIGHTS

- *ASIC1*^{-/-} mice have longer latency uncovering buried food
- *ASIC1*^{-/-} mice have reduced odor sensitivity to acidic compounds
- Nasal administration of ASIC1 inhibitor PcTX1 increases the latency for uncovering buried food

Figure 1. Buried Food Test in WT and ASIC1^{-/-} Mice

1A. The latency for WT (n=8) and ASIC1^{-/-} (n=9) mice to locate and uncover buried food. Latency was measured as time (s) spent by the animals to uncover buried food within 600 sec. In trial 1, a significant difference was observed between two groups ($p < 0.01$). 1B. Overall performance in buried food and surface pellet tests. The average latency of ASIC1^{-/-} mice was significantly longer compared to WT mice ($p < 0.01$). Compared to WT mice, ASIC1^{-/-} mice also spent longer time locating visible food ($p < 0.05$).

Figure 2. Olfactory Habituation/Dishabituation Test

The ability of WT (n=8) and ASIC1^{-/-} (n=7) mice to recognize different odors was examined. Investigation time was measured as time (s) spent by the animal sniffing odorwet cotton swabs (water, orange extract and acetic acid) during three consecutive 2 minutes of each odor (T1, T2, T3). A. The investigation time (s) by WT and ASIC1^{-/-} mice sniffing water. B–C. The investigation time (s) spent by WT and ASIC1^{-/-} mice sniffing orange or acetic acid. The initial exposure time of ASIC1^{-/-} mice for orange extract ($p<0.01$) and acetic acid ($p<0.01$) was significantly longer than WT mice.

Figure 3.

Olfactory Preference Test of Olfactory Attraction/avoidance Behavior. WT (n=6) and ASIC1^{-/-} (n=6) mice's investigation time for an attractant and aversive odorant was measured. 3A. No difference in investigation time was observed between WT and ASIC1^{-/-} mice during exposure to peanut butter extract. 3B. ASIC1^{-/-} mice have longer investigation time during exposure to 8% TMT compared to WT, $p < 0.05$.

Figure 4.

Effect of Zinc Gluconate Treatment on BFT. Latency (s) for sham (n=3) and zinc gluconate-treated (n=4) mice. Testing was performed an hour after treatment. Zinc gluconate-treated mice had greater latency uncovering buried food following the treatment, $p < 0.05$.

Figure 5. Effect of PcTX1 Treatment on BFT. Latency (s) for sham (n=4) and PcTX1-treated mice (n=4). Testing was performed an hour after treatment. PcTX1 treated mice had longer latency uncovering buried food following treatment, $p < 0.05$.